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Understanding microoxygenation: Effect of viable yeasts and sulfur dioxide levels on the sensory properties of a Merlot red wine

María-Pilar Sáenz-Navajas[a], Charlie Henschen[b], Annegret Cantu[b], Aude A. Watrelot[b], Andrew L. Waterhouse[b,*]

[a] Laboratorio de Análisis del Aroma y Enología (LAAE), Department of Analytical Chemistry, Universidad de Zaragoza (UZ), Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), Associate unit to Instituto de las Ciencias de la Vid y del Vino -ICVV- (UR-CSIC-GR), Zaragoza, Spain
[b] Department of Viticulture and Enology, University of California, Davis, CA, United States

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ABSTRACT

Microoxygenation (Mox) is widely used in winemaking to improve color, in-mouth properties and aroma, but its use is not always predictable. Here we investigate the effect of Mox, (while monitoring viable yeasts and SO_2 levels), on color, anthocyanin-derived pigments, tannins, aroma and in-mouth sensory properties as well as on hedonic rating by wine experts. Results on this Merlot wine show that the re-appearance of viable *Saccharomyces cerevisiae* yeasts modulates oxygen consumption rates, and dramatically increases acetaldehyde levels. This led to significant sensory changes, particularly for aroma. Mox reduced green-vegetable and reduction-related aromas, but also astringent mouth-feel properties related to tannins, and lower astringency was correlated with lower tannin activity. The Mox wines that exhibited yeast growth had higher hedonic scores from one group of expert tasters based on increased jammy/dried fruit flavors, while another group of tasters rated the non-Mox wines higher due to the green vegetable and spicy aromas. These results show that the chemical and sensory impact of a Mox treatment is highly dependent on the absence or presence of yeast growth, so it is important to monitor for yeast populations during Mox treatment.

1. Introduction

Microoxygenation (Mox) is a winemaking tool that consists in supplying controlled levels of oxygen to generate desired sensory effects on the product. It is commonly employed at different stages of the winemaking process, depending on the goal of the enologist. The application of Mox during alcoholic fermentation is aimed at helping the yeast produce membrane lipids, which results in an increase in yeast viability and ethanol tolerance (Valero, Millán, & Ortega, 2001). The main goals of applying Mox after alcoholic fermentation, both before and after malolactic fermentation (MLF) is to stabilize color, decrease bitterness (Cejudo-Bastante, Pérez-Coello, & Hermosín-Gutiérrez, 2011) and modify aroma properties.

Mox increases the levels of acetaldehyde, which is involved in the formation of stable pigments such as anthocyanin-ethyl-flavanol derivatives or pyranoanthocyanins (Vivar-Quintana, Santos-Buelga, Francia-Aricha, & Rivas-Gonzalo, 1999). These stable pigments are more resistant to bleaching than native anthocyanins (Fulcrand, Atanasova, Salas, & Cheynier, 2004). Acetaldehyde also induces the polymerization of flavanols, which are involved in the perception of bitterness and astringency. The formation of these condensation products decreases bitterness, while the effect on astringency seems to be wine-dependent. This mouthfeel sensation has been described to increase in Cencibel (Cejudo-Bastante, Hermosín-Gutiérrez, & Pérez-Coello, 2011; Cejudo-Bastante, Pérez-Coello, et al., 2011), decrease in Merlot (Cejudo-Bastante, Hermosín-Gutiérrez, et al., 2011) or even remain unaltered in Tempranillo wines when Mox is applied before MLF (Sanchez-Iglesias, Gonzalez-Sanjose, Perez-Magarino, Ortega-Heras, & Gonzalez-Huerta, 2009). Similarly, for Mox after MLF, the results seem to be contradictory as the intensity of astringency has shown to decrease in Cabernet Sauvignon (Parpinello, Plumejeau, Maury, & Versari, 2012) and Cencibel red wines (Cejudo-Bastante, Hermosín-Gutiérrez, & Pérez-Coello, 2012), while in a blend of Cabernet Sauvignon, Merlot and Malbec a loss of mouthfeel has been reported (Oberholster et al., 2015).

With regard to aroma changes, there are reports showing a loss of reductive aroma in Cabernet Sauvignon (McCord, 2003) and of herbaceous nuances in Cencibel (Cejudo-Bastante et al., 2012; Cejudo-Bastante, Pérez-Coello, et al., 2011), while Oberholster et al. (2015) reported increases in vegetative aromas. These results show that it is

* Corresponding author.
E-mail address: alwaterhouse@ucdavis.edu (A.L. Waterhouse).

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very difficult to predict the effect of Mox on wine properties. This is likely related to poorly controlled factors involved in the Mox process, leading to highly variable and contradictory outcomes.

The main goal of applying Mox after MLF is to induce an accelerated aging of the wine that allows an earlier release of the product to the market. The process must be controlled to avoid over-oxidizing treated wines, resulting in sensory defects. Chemical oxidation of wine largely results in the oxidation of ethanol to acetaldehyde. The rate of this reaction is mainly dependent on the concentration of sulfur dioxide (Tao, Dykes, & Kilmartin, 2007) or other antioxidants such as glutathione (Gambuti, Han, Peterson, & Waterhouse, 2015) and the level of reactive polyphenols available in wine (Picariello, Gambuti, Picariello, & Moio, 2017). Under ideal conditions, all oxidation (O_2) ultimately reacts with SO_2, resulting in a stoichiometric mass ratio of 4:1, however in real conditions wines do not always obey to this ratio. In some cases, this could be attributed to the presence of other antioxidants different from SO_2. However, it is possible that some dormant yeasts from the alcoholic fermentation can survive and activate in the presence of oxygen. This process might be related to that observed in wines aged *sur lies*. These wines consume oxygen faster due to the presence of nonviable yeasts, which compete with phenols and slow down the aging process (Salmon, 2006; Salmon, Fornairon-Bonnefond, & Mazauric, 2002). In this context, it is hypothesized that after MLF, there are still some dormant, but biologically active microorganisms (yeasts or bacteria) that can grow in the presence of the controlled amounts of oxygen during Mox, and they would utilize this oxygen to increase acetaldehyde production. If this is the case, the growth of microorganisms would be a key factor to be controlled during Mox. Thus, it can be secondly hypothesized that the development of yeasts and/or bacteria would induce sensory differences compared to wines that do not develop these microorganisms.

So, the present work aims to study 1) the effect of sterile filtration on dissolved oxygen and yeast growth during Mox treatment, and 2) the effect of yeast development on color, acetaldehyde, anthocyanin-derivative pigments, tannins, sensory properties, and hedonic ratings.

2. Material and methods

2.1. Wine

Five-hundred liters of red wine made with Merlot grapes (100%) and produced in 2015 was supplied by Constellation Brands. After malolactic fermentation (MLF), the wine had been centrifuged, but it has not been treated with Mox prior to delivery to UC Davis. Conventional enological parameters of the initial wine are given in Table 1.

2.2. Experimental design

A total of 16 sterilized stainless steel tanks with a capacity of 23 L were employed. Half of them were submitted to microoxygenation (Mox) during 48 days at a rate of 15 mg L^{-1} $month^{-1}$ and half of them not treated (NMox), which served as controls. Each set of Mox and NMox treatments was formed by eight tanks. Four of them were microfiltered through 0.22 µm (F) to remove residual yeasts and/or bacteria and four of them were unfiltered (UF) with the aim of evaluating the effect of residual viable yeasts on the treatments. Further, the effect of the level of sulfur dioxide (SO_2) was evaluated by adding two levels of SO_2 and finally obtain high (average: 40.2 ± 1.6 mg L^{-1}) and low (average: 22.3 ± 1.6 mg L^{-1}) levels of free SO_2. Sulfur dioxide was added only once at the beginning of the experiment. Table 2 shows the codes and treatments for each of the 16 tanks.

Table 1
Conventional enological parameters of wine at the start of the experiment.

Parameter	Concentration
Ethanol (% v/v)	14.2
pH	3.60
Titratable acidity (g L^{-1})[a]	5.3
Residual sugar (g L^{-1})	3.0
Malic acid (g L^{-1})	0.01
Volatile acidity (g L^{-1})[b]	0.60
Free SO_2 (mg L^{-1})	23.8
total SO_2 (mg L^{-1})	51.3

[a] Expressed as g L^{-1} of tartaric acid.
[b] Expressed as g L^{-1} of acetic acid.

2.2.1. Microoxygenation system

Controlled amounts of oxygen were added to eight purpose-built tanks (Gambuti et al., 2015). Briefly, each tank had a three-point entry together with an exit for oxygen. The first port was a sampling device, the second a dissolved oxygen sensor and in the third a sterile entry for the oxygen delivery system. Oxygen was delivered under pressure by means of a fluorinated ethylene propylene tubing FEP-188x250 (Ozone solutions, Inc., Hull, IA). With the tanks closed, dissolved oxygen (DO) was measured daily by means of an oxygen sensor (PreSens PST3, Nomacorc LCC, Zebulon, NC). All tanks were kept at a temperature of 19.5 ± 0.1 °C and they were continuously stirred to avoid DO gradients.

Wines were sampled at the beginning (t = 0 days) of the experiment and at 7, 13, 20, 27, 32 and 39 days after Mox began, to measure free and total SO_2, acetaldehyde, and microbiological growth. Samples collected at the end (t = 48 da) of the treatment were evaluated for anthocyanins, pigmented derivatives, color and tannins (concentration and activity). The Mox experiment finished in April 2016 and was carried out in the University of California at the Department of Viticulture and Enology (Davis, USA).

2.2.2. Bottling

Wines were filtered and bottled 20 days after the end of the treatment (t = 48 da). Each tank was supplemented with potassium metabisulfite before bottling to achieve a final concentration of free SO_2 of 10 mg L^{-1}. All wines were filtered in-line immediately prior to bottling through 0.45 µm-cartridges. Glass bottles were dosed with liquid nitrogen after filling and closed with Diam10 cork closures (Diam, Céret, France).

2.3. Sensory analysis

First, a pretest (flash profile) was carried out with the aim of reducing the number of wines to be evaluated in further formal sensory tasting. Selected samples were then submitted to a sorting task by a panel of experts.

In both tasks, all samples were presented simultaneously in a random order that was different for each assessor. Twenty-mL samples were poured in clear wine glasses (ISO NORM 3591, 1977) labelled with 3-digit random codes and covered by plastic Petri dishes. All samples were served at room temperature and evaluated in individual booths. Panelists were not informed about the nature of the samples to be evaluated.

2.3.1. Pretest. Selection of wine samples

2.3.1.1. Participants. Four wine experts (2 women and 2 men) from the department of Enology and Viticulture of UC Davis completed one two-hour session.

2.3.1.2. Samples. All sixteen samples were evaluated one week after bottling (i.e. one month after Mox treatment was finished).

2.3.1.3. Procedure. The procedure consisted of flash profile involving